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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application

Inventor(s): Woudenberg, et al.

Application No.: 08/235,411

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Title: SYSTEM FOR REAL TIME
DETECTION OF NUCLEIC ACID
AMPLIFICATION PRODUCTS

PATENT APPLICATION

Group Art Unit: 1807

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DECLARATION UNDER 37 C.F.R. § 1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Will Bloch, declare that the following is true and correct:

1. I am a member of the Human Genetic Analysis Group within the Applied Biosystems Division of Perkin Elmer and presently hold the title of Senior Staff Scientist. I have been with Applied Biosystems since 1992.
2. I am familiar with the subject matter which is being claimed in U.S. Patent Application Serial No.: 08/235,411, entitled: SYSTEM FOR REAL TIME DETECTION OF NUCLEIC ACID AMPLIFICATION PRODUCTS which was filed on April 29, 1994. A copy of claims which I understand are being pursued is attached hereto as Exhibit 1.
3. Linda G. Lee and Charles L. Connell, are named as co-inventors of the above-referenced patent application and are listed as co-authors of Lee, et al., Nucleic Acids Research, 21:3761-3766 (1993).
4. I am also a co-author of Lee, et al., Nucleic Acids Research, 21:3761-3766 (1993). Although I am listed as a co-author of Lee, et al., I did not contribute to the

inventive subject matter described in Lee, et al. which is presently being claimed in the above-referenced application.

5. I make this declaration with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001) and may jeopardize the validity of the patent. All statements of my own knowledge are true. I believe all statements made herein on information and belief to be true.

Executed this 15th day of March, 1996 in Foster City, California.


Will Bloch



**EXHIBIT 1 TO DECLARATION
BY WILL BLOCH**

1. An apparatus for monitoring the formation of a nucleic acid amplification reaction product in real time, the apparatus comprising:
 - a sample holder for holding a sample of nucleic acids to be amplified;
 - a fiber optic cable for illuminating a volume of the sample with an excitation beam;
 - a lens co-axially disposed with the fiber optic cable for focusing the excitation beam into the volume of the sample, the lens collecting from the sample and transmitting to the fiber optic cable a first fluorescent signal whose intensity is proportional to the concentration of the amplification reaction product in the volume of sample illuminated by the excitation beam and a second fluorescent signal whose intensity is proportional to the volume of the sample illuminated by the excitation beam; and
 - a detection and analysis mechanism for receiving the first and second fluorescent signals from the fiber optic cable.
2. The apparatus according to claim 1 wherein the first and second fluorescent signals each have an intensity and the detection and analysis mechanism provides a readout including a ratio between the intensity of the first fluorescent signal and the intensity of the second fluorescent signal.
3. The apparatus according to claim 1 wherein the apparatus includes
 - a plurality of sample holders for holding a plurality of samples,
 - a plurality of fiber optic cables for illuminating volumes of the plurality of samples,
 - a plurality of lenses, each co-axially disposed with a first end of a fiber optic cable for focusing an excitation beam into a sample, and
 - a fiber optic multiplexer which couples the detection and analysis mechanism to a second end of each of the plurality of fiber optic cables.
4. The apparatus according to claim 1 wherein the sample holder includes a removable reaction chamber for holding the sample.

5. The apparatus according to claim 1 wherein the removable reaction chamber is sealable.
6. The apparatus according to claim 1 wherein the sample holder includes a sealable reaction chamber for holding the sample.
7. The apparatus according to claim 1 wherein the sample holder includes an optical interface through which the excitation beam is transmitted from the lens into the sample.
8. The apparatus according to claim 7 wherein the sample holder includes a sealable reaction chamber for holding the sample, the optical interface forming a wall of the reaction chamber.
9. The apparatus according to claim 7 wherein the apparatus further includes a mechanism for heating the optical interface to prevent condensation of the sample on the optical interface.
10. The apparatus according to claim 9 wherein the sample holder includes a sealable reaction chamber for holding the sample, the optical interface forming a wall of the reaction chamber.
11. The apparatus according to claim 7 wherein the sample holder includes a removable reaction chamber for holding the sample, the optical interface forming a wall of the reaction chamber which covers at least a portion of the sample and which is separated from the sample by an air gap.
12. A method for monitoring the formation of a nucleic acid amplification reaction product in real time comprising:
 - adding a sample to a sample holder which contains a nucleic acid sequence to be amplified,
 - transmitting an excitation beam into the sample which illuminates a volume of the sample, the sample including a first fluorescent indicator which produces a first fluorescent

signal when illuminated by the excitation beam whose intensity is proportional to the concentration of amplification reaction product in the sample and the volume of the sample illuminated by the excitation beam, and a second fluorescent indicator homogeneously distributed throughout the sample which produces a second fluorescent signal when illuminated by the excitation beam whose intensity is proportional to the volume of the sample illuminated by the excitation beam; and

measuring the intensities of the first and second fluorescent signals.

13. The method according to claim 12 wherein the first and second fluorescent signals each have an intensity and the detection, the step of measuring the intensities of the first and second fluorescent signals including calculating a ratio between the intensity of the first fluorescent signal and the intensity of the second fluorescent signal.

14. The method according to claim 12 wherein the first fluorescent indicator is a complex-forming dye.

15. The method according to claim 12, further including the step of sealing the sample within the sample holder prior to transmitting an excitation beam into the sample.

16. The method according to claim 12 wherein the sample holder includes an optical interface through which the excitation beam is transmitted from the lens to the sample, the sample holder also including an air gap separating the optical interface from the sample, the method further including the step of heating the optical interface to prevent condensation of the sample on the optical interface.

17. The method according to claim 16, further including the step of sealing the sample within the sample holder prior to transmitting an excitation beam into the sample.

18. The method according to claim 24 wherein the step of adding a sample to a sample holder includes

adding a sample to a reaction chamber which is removable from the sample holder; and
adding the removable reaction chamber to the sample holder.

19. The method according to claim 18, further including the step of sealing the sample within the removable reaction chamber.

20. The method according to claim 18 wherein the removable reaction chamber includes an optical interface through which the excitation beam is transmitted from the lens to the sample and an air gap separating the optical interface from the sample, the method further including the step of heating the optical interface to prevent condensation of the sample on the optical interface.

21. The method according to claim 12 wherein the nucleic acid amplification reaction is a polymerase chain reaction.

22. The method according to claim 12 wherein the nucleic acid amplification reaction is a ligase chain reaction.

23. The method according to claim 12 wherein the nucleic acid amplification reaction is a polymerase chain reaction and wherein the first and second fluorescent indicators are covalently attached to an oligonucleotide having a nucleotide sequence complementary to a portion of a strand of the amplification reaction product, the second fluorescent indicator quenching the fluorescence of the first fluorescent indicator.

24. A method for monitoring the formation of nucleic acid amplification reaction products in a plurality of samples in real time comprising:

adding samples containing a nucleic acid sequence to be amplified to a plurality of sample holders;

transmitting excitation beams into the plurality of sample holders which illuminate a volume of each sample, each sample including a first fluorescent indicator which produces a first fluorescent signal when illuminated by the excitation beam whose intensity is proportional to the concentration of amplification reaction product in the sample and the volume of the sample illuminated by the excitation beam, and a second fluorescent indicator homogeneously distributed throughout the sample which produces a second fluorescent signal when illuminated

by the excitation beam whose intensity is proportional to the volume of the sample illuminated by the excitation beam; and

measuring the intensities of the first and second fluorescent signals of each of the samples.

25. The method according to claim 24 wherein at least two different first fluorescent indicators having different first fluorescent signals are used amongst the plurality of samples, the step of measuring the intensity of the first fluorescent signal including measuring the different first fluorescent signals of the at least two different first fluorescent indicators.

26. A method for monitoring the formation of a plurality of nucleic acid amplification reaction products in a sample in real time comprising:

adding to a sample holder a sample containing a plurality of different nucleic acid sequences to be amplified,

transmitting an excitation beam into the sample which illuminates a volume of the sample, the sample including a plurality of first fluorescent indicators which each produce a first fluorescent signal when illuminated by the excitation beam whose intensity is proportional to the concentration of a particular amplification reaction product in the sample and the volume of the sample illuminated by the excitation beam, and a second fluorescent indicator homogeneously distributed throughout the sample which produces a second fluorescent signal when illuminated by the excitation beam whose intensity is proportional to the volume of the sample illuminated by the excitation beam; and

measuring the intensities of the first and second fluorescent signals.